L2: Entry 373 of 425 File: USPT Feb 2, 1999

US-PAT-NO: 5866132
DOCUMENT-IDENTIFIER: US 5866132 A

TITLE: Immunogenic oligosaccharide compositions

DATE-ISSUED: February 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 424/193.1; 424/234.1, 424/243.1, 530/395, 530/403, 530/405

CLAIMS:

What is claimed is:

1. A composition comprising a conjugate, wherein each conjugate consists essentially of:

(a) at least one oligosaccharide hapten which retains at least one immunogenic epitope wherein each said oligosaccharide hapten has a multiple of repeat subunits; and

(b) a carrier which elicits a thymus dependent immune response in a subject, wherein said hapten is covalently coupled directly to said carrier and wherein said hapten-carrier conjugate is protectively immunogenic.

2. The composition of claim 1 wherein said hapten is an oligosaccharide of a bacterial or viral polysaccharide.

3. The composition of claim 1 wherein the presence of said immunogenic epitope is determined using inhibition ELISA.

4. The composition of claim 2 wherein said oligosaccharide is produced by acid hydrolysis of said polysaccharide.

5. The composition of claim 1 wherein said protective immunogenicity is determined by isotype ELISA.

6. The composition of claim 1 wherein said protective immunogenicity is determined by bactericidal or opsonization assay.

7. The composition of claim 2 wherein said polysaccharide is selected from the

group consisting of capsular polysaccharides of S. pneumococcus serotypes 1, 2, 3, 4, 5, 6B, 7, 7F, 8, 9N, 9V, 10A, 11A, 12, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22, 23F and 33F.

8. The composition of claim 1 which comprises two or more haptens.

9. The composition of claim 1 which does not induce carrier suppression.

10. The composition of claim 1 which does not induce antigenic competition.

11. The composition of claim 1 further comprising an adjuvant.

12. A composition comprising:

(a) a conjugate which comprises a size-separated oligosaccharide of S. pneumoniae serotype 8 which retains an immunogenic epitope which oligosaccharide is directly coupled to a protein carrier which elicits a thymus dependent immune response in a subject; and

(b) a suitable pharmaceutical excipient, wherein said conjugate provides an immunoprotective effect.

13. The composition of claim 12 which does not induce carrier suppression.

14. The composition of claim 12 which does not induce antigenic competition .

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L4: Entry 207 of 215 File: USPT Apr 22, 1997

US-PAT-NO: 5623057
DOCUMENT-IDENTIFIER: US 5623057 A

TITLE: Pneumococcal polysaccharide conjugate vaccine

DATE-ISSUED: April 22, 1997

INVENTOR-INFORMATION:

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US-CL-CURRENT: 530/404; 424/193.1, 424/194.1, 424/197.11, 424/234.1, 424/237.1, 424/241.1, 424/244.1, 424/256.1, 424/260.1, 530/403, 530/405, 530/406, 530/408, 530/409

CLAIMS:

what is claimed is:

1. A conjugate comprising an immunogenic protein selected from OMPC and MIEP covalently linked to a polysaccharide derived from one or more subtypes of Streptococcus pneumoniae, said polysaccharide having, on average, between 60 and 1200 repeating units per molecule and a polydispersity between 1.0 and 1.4, wherein said polysaccharide has a molecular weight between, on average, 1.times.10.sup.5 and 1.times.10.sup.6, and a level of contamination by pneumococcal group-specific C-polysaccharide below 3.0% of the type-specific polysaccharide.

2. The conjugate of claim 1 wherein said polysaccharide has an antigenicity index between 0.4 and 1.1.

3. The conjugate of claim 2 wherein said polysaccharide has an intrinsic viscosity between 0.6 and 3.0 dL/g and an antigenicity index of between 0.7 and 1.1.

4. The conjugate of claim 3 wherein said polysaccharide is derived from any of the subtypes of Streptococcus pneumoniae selected from: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F.

5. The conjugate of claim 4 wherein said polysaccharide is derived from between one and all of the capsular polysaccharides from Streptococcus pneumoniae subtype: 4, 6B, 9V, 14, 18C, 19F, or 23F.

6. The conjugate of claim 5 wherein said polysaccharide has a size polydispersity between 1.0 and 1.4, more than 60 repeating units per molecule, a C-polysaccharide contamination level as compared with type specific polysaccharide of less than 3%, and is derived from:

1) Streptococcus pneumonias 6B, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.60.+-.0.05;

c) a M.sub.W between about 3.times.10.sup.5 and 7.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 1000 repeating units per molecule on average;

2) Streptococcus pneumoniae 14, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 8.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.60.+-.0.05;

c) a M.sub.W between about 4.times.10.sup.5 and 1.times.10.sup.6 ; and

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 0.6 and 1.6; and

e) less than about 1200 repeating units per molecule on average;

3) Streptococcus pneumoniae 19F, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 1000 monomer repeating units per molecule, on average;

4) Streptococcus pneumonias 23F, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.54.+-.0.05;

c) a M.sub.W between about 4.times.10.sup.5 and 8.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.5 and 3.0; and

e) less than about 1000 monomer repeating units per molecule, on average;

5) Streptococcus pneumoniae 4, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 4.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 5.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 3.0; and

e) less than about 600 monomer repeating units per molecule, on average;

6) Streptococcus pneumonias 9V, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 3.times.10.sup.5 and 7.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 800 monomer repeating units per molecule, on average; or

7) Streptococcus pneumonias 18C, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.5 and 3.0. and

e) less than about 700 monomer repeating units per molecule, on average; or a mixture of any of these polysaccharides.

7. The covalent conjugate of claim 6 wherein the OMPC or MIEP and the Pn-Ps are linked through a spacer as shown by the formula: ##STR28## for linkagages through the polysaccharide hydroxyls, or ##STR29## in the case of polysaccharides bearing unblocked carboxyllic acid groups, wherein PRO represents OMPC or MIEP, and Pn-Ps represents a penumococcal polysaccharide.

8. The conjugate of claim 7 wherein the conjugate has a Pn-Ps:OMPC, or Pn-Ps:MIEP mass ratio between about 0.05 and 0.5, and upon hydrolysis and amino acid analysis yields a SCMHC/Lys ratio between 0.01 and 0.15.

9. A pneumococcal polysaccharide-immunogenic protein conjugate produced by the process of:

(a) Culturing a pneumococcus and isolating crude pneumococcal polysaccharide or solubilizing pneumococcal polysaccharide powder;

(b) Purifying and partially-hydrolyzing the polysaccharide of step (a) to an endpoint predetermined to generate a polysaccharide amenable to conjugation having no more than a 30% reduction of the polysaccharide's type-specific antigenicity as compared with the crude polysaccharide of step (a); and

(c) Conjugating the product of step (b) with OMPC or MIEP; wherein the pneumococcus cultured in step (a) is selected from one or more of the subtypes: 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, and further, wherein the Pn-Ps retains its antigenic integrity as measured by Ouchterlony double immunodiffusion or rate nephelometry assay using an anti-Pn-Ps type-specific antibody, said Pn-Ps prior to conjugation being physically sheared in a Gaulin press at a pressure between about 2000 and 15000 PSI or hydrolyzed by heating at 100.degree. C. for 24 hours or by sonicating, to a viscosity for a 1 mg/ml solution in 0.9M sodium chloride or K.sub.d (peak) endpoint as follows for each listed Pn-Ps subtype:

Pn-Ps Subtype	Target Endpoint Viscosity (centistokes)	Target Endpoint K.sub.d (peak)
Pn4-Ps	1.5-1.00	0.65 .+-. 0.05
Pn6B-Ps	1.3-1.00	0.60 .+-. 0.05
Pn6B-Ps	1.3-1.00	0.60 .+-. 0.05
Pn9V-Ps	1.3-1.00	0.65 .+-. 0.05
Pn14-Ps	1.1-0.95	0.60 .+-. 0.05
Pn18C-Ps	1.5-1.00	0.65 .+-. 0.05
Pn19F-Ps	1.3-1.00	0.65 .+-. 0.05
Pn23F-Ps	1.5-1.00	0.54 .+-. 0.05;

optionally followed by chromatographic or alcohol fractionation to select material having a polydispersity below 1.4.

10. A process for making a Pn-Ps-PRO conjugate which comprises:

a) Isolating crude pneumococcal polysaccharide, Pn-Ps;

b) i-Optionally purifying the crude Pn-Ps by ion exchange adsorption of impurities; ii-Partially-hydrolyzing or mechanically-shearing the crude Pn-Ps;

Fractionating the partially-hydrolyzed Pn-Ps according to size and purity;

d) Derivatizing the fractionated Pn-Ps, derived from one or more pneumococcal subtypes according to steps (a)-(c), to display pendant nucleophilic or electrophilic moieties;

e) Isolating Neisseria meningitidis b OMPC, or MIEP;

f) Functionalizing the OMPC or MIEP to exhibit reactive electrophilic or nucleophilic moieties;

g) Conjugating the polysaccharide of step (d) with the protein of step (f);

h) Capping the conjugate to remove residual functional groups; and

i) Isolating the conjugate product, wherein steps (b) and (c) further comprise:

(b) 1-Optionally, adsorbing onto Whatman DE52 anionic impurities at a solution pH of about 5; 2-Partially hydrolyzing the Pn-Ps in solution to an endpoint viscosity predetermined to diminish the Pn-Ps binding to anti-pneumococcal type specific antibody by no more than 30% as compared with crude Pn-Ps by:

1. heating at 50.degree. to 150.degree. C. for between 1 to 48 hours; or

2. sonicating for periods of 5 seconds to 5 minutes, depending on the power setting of the sonication probe, followed by periods of cooling and additional sonication; or

3. shearing in a Gaulin press at pressures between about 2000 and 15000 PSI; and

c) Fractionating the partially-hydrolyzed Pn-Ps according to size and purity wherein step (c) comprises:

Fractionating the hydrolyzed Pn-Ps and selecting a fraction having a molecular weight in the range between 1.times.10.sup.5 and 1.times.10.sup.6 by:

i-differential alcohol solubility using isopropanol at concentrations predetermined to precipitate the desired Pn-Ps size range, or

ii-fractionation on a size-exclusion liquid chromatography column capable of including and fractionating polysaccharides in the size range between 5.times.10.sup.4 and 1.times.10.sup.6 wherein the

endpoint for hydrolysis or shear is determined by viscometry of a 1 mg/ml solution in 0.1M sodium phosphate, pH 7.2, or chromatography for each of the listed polysaccharides according to the end-point for that subtype Pn-Ps:

Pn-Ps Subtype	Target Endpoint Viscosity (centistokes)	Target Endpoint K.sub.d (peak)
Pn4-Ps	1.5-1.00	0.65 .+-. 0.05
Pn6B-Ps	1.3-1.00	0.60 .+-. 0.05
Pn9V-Ps	1.3-1.00	0.65 .+-. 0.05
Pn14-Ps	1.1-0.95	0.60 .+-. 0.05
Pn18C-Ps	1.5-1.00	0.65 .+-. 0.05
Pn19F-Ps	1.3-1.00	0.65 .+-. 0.05
Pn23F-Ps	1.5-1.00	0.54 .+-. 0.05.

L4: Entry 207 of 215 File: USPT Apr 22, 1997

DOCUMENT-IDENTIFIER: US 5623057 A
TITLE: Pneumococcal polysaccharide conjugate vaccine

Abstract Text (1):
A novel conjugate vaccine comprising partially hydrolyzed, highly purified, capsular polysaccharide (Ps) from Streptococcus pneumoniae bacteria (pneumococci, Pn) linked to an immunogenic carrier protein, is produced by a new process. The conjugate is useful in the prevention of pneumococcal infections. Vaccines comprising a mixture of from one to ten different pneumococcal polysaccharide-immunogenic protein (Pn-Ps-PRO) conjugates induce broadly protective recipient immune responses against the cognate pathogens from which the polysaccharide components are derived. Young children and infants younger than 2 years old, normally unable to mount a protective immune response to the Pn-Ps alone, exhibit protective immune responses upon vaccination with these Pn-Ps-PRO conjugates.

Brief Summary Text (2):
The pathogenic bacteria classified as Streptococcus pneumoniae (pneumococci, Pn) have been subdivided into 84 antigenic serotypes, based on the capsular polysaccharide (Pn-Ps) of the organism. Disease states attributable to these organisms include pneumonia, meningitis, otitis media, bacteremia and acute exacerbations of chronic bronchitis, sinusitis, arthritis and conjuctivitis. The preponderance of these diseases, however, are caused by a limited subset of the 84 known isolates. Thus a polyvalent vaccine containing the Pn-Ps from the most prevalent and pathogenic isolates of the organism can provide protection against a very high percentage of the most frequently reported pathogens of this class.

Brief Summary Text (3):
Polyvalent vaccines have been produced that are efficacious in raising protective immune responses against the pneumococci in adults. "PNEUMOVAX.RTM. 23" (Pneumococcal Vaccine Polyvalent, MSD; see PDR, 1990 edition, p. 1431), for example, is a liquid composition containing 50 .mu.g/ml of each of the 23 different, unconjugated pneumococcal polysaccharides, all of which are on deposit with the ATCC and provid one

possible source of starting material for this invention. "PNEUMOVAX.RTM. 23" comprises each of the following free, that is unconjugated, polysaccharides: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F, accounting for about 90% of pneumococcal blood isolates. However, such vaccines are least effective in the segment of the population most at risk for pneumococcal infections: B-Cell immunocompromised individuals, the elderly and infants younger than two years old who depend on T-cell responses for immune protection. Since unconjugated polysaccharides are poor inducers of T-cell immune responses, conversion of the Pn-Ps into immunogens capable of inducing T-cell responses is the key to producing adequate protection in this target population. Use, however, is not restricted to this group of individuals. For example, administration of a vaccine, comprising one or more of the novel conjugates, to a female mammal prior to or during pregnancy raises antibodies in the mother which can passively protect a developing fetus and suckling infant even though the vaccine is not administered directly to the fetus or infant. Such conjugate vaccines should also prove useful for eliciting antibodies for ultimate passive protection of at risk populations, such as newborns or siblings of infected individuals.

Brief Summary Text (7):
In addition to the novel conjugate product, this invention discloses a new process for preparing partially hydrolyzed, highly purified pneumococcal polysaccharide intermediates, novel compositions comprising from one to ten different conjugates and methods of using the invention. Of particular interest are the capsular polysaccharides included in "PNEUMOVAX.RTM. 23" (Pneumococcal Vaccine Polyvalent, MSD; see PDR, 1990 edition, p. 1431). A most preferred subset are the capsular polysaccharides of Streptococcus pneumoniae subtypes 6B, 23F, 19F, 14, 18C, 4 and 9V, as this small group of pneumococcal subtypes are estimated to be responsible for between 75-85% of pneumococcal infections in infants and children. The methods provided herein are applicable to a broad collection of pneumococcal and other bacterial polysaccharides.

Brief Summary Text (10):
Novel and highly antigenic Pn-Ps-PRO conjugates of the invention, comprising the outer membrane protein complex (OMPC) or Neisseria meningitidis b, or recombinant or purified subunits thereof, such as MIEP, or other immunogenic carrier proteins covalently linked to partially hydrolyzed and highly purified Pn-Ps intermediates from prevalent pneumococcal isolates, are useful for prevention of pneumococcal infections in mammals. The conjugates are particularly useful in vaccine compositions for stimulating anti-pneumococcal immune responses in mammals, especially in B-cell immunocompromised individuals, the elderly and in human infants younger than two years old, as the conjugates elicit T-cell responses. Pn-Ps-OMPC and Pn-Ps-MIEP conjugates are made by a process comprising the steps of: isolating capsular Ps from cultures of Streptococcus

pneumoniae (pneumococci, Pn), partially hydrolyzing or physically shearing the Pn-Ps, fractionating said Pn-Ps, to yield a Pn-Ps product of reduced molecular size, polydispersity, viscosity and then covalently conjugating the Pn-Ps to OMPC or MIEP.

Brief Summary Text (17):
The novel partially hydrolyzed and highly purified pneumococcal capsular polysaccharide (Pn-Ps) is a preparation of an antigenic polysaccharide derived from a culture of one of the pneumococcal subtypes (as described below in the section describing a novel conjugation process and in Examples 3-10). The Pn-Ps has an average molecular weight between about 1.times.10.sup.5 and 1.times.10.sup.6 daltons, on average less than about 1000 repeating units per molecule, a C-polysaccharide contamination level of less than about 3% and an antigenicity index between 0.4 and 1.1, and preferably between 0.7 and 1.1. This last parameter is the relative amount of anti-pneumococcal type-specific antibody binding exhibited per unit mass of the new Pn-Ps as compared with crude Pn-Ps on deposit with the ATCC. Furthermore, the novel Pn-Ps is amenable to conjugation with immunogenic protein to produce the Pn-Ps-PRO product of the invention. Some physical and chemical characteristics of 2 different Pn6B-Ps and 2 different Pn23F-Ps preparations are given in Table I below, while the description that follows reveals how those characteristics are measured. The process disclosed below provides a method for making Pn-Ps intermediates and Pn-Ps-PRO conjugates with a wide variety of pneumococcal subtypes, including but not restricted to those selected from subtypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F. As mentioned above, a preferred subset of pneumococcal polysaccharides are those derived from pneumococcal subtypes 4, 6B, 9V, 14, 18C, 19F and 23F. It wil be obvious to those skilled in the art that in addition to or in place of any of these polysaccharides, others may be substituted as the need arises in the at-risk polulation. Thus, Pn1-Ps and Pn5-Ps may be treated like Pn4-Ps or Pn9V-Ps, as could Neisseria meningitidis B, C, or Group B Streptococcal polysaccharides, while Pn7F-Ps may be treated like Pn14-Ps, as further described below, and included in a multivalent vaccine. It will also be clear to those skilled in the art that by inclusion of Pn6B-Ps, protection against Pn6A would be provided by cross-reactive antibodies. This is also true for a number of other pneumococcal subtypes.

Brief Summary Text (18):
Multivalent vaccines are those comprising mixtures of different Pn-Ps-PRO conjugates each prepared separately with a given Pn-Ps subtype. In addition, multivalent vaccines are those wherein several different Pn-Ps subtypes are all conjugated to a given PRO at one time or sequentially.

Detailed Description Text (41):

Culturing Streptococcus pneumoniae subtypes and Isolation of Crude Pn-Ps:

Detailed Description Text (44):
The growth on the plate is resuspended in Heart Infusion Broth and an aliquot of the resuspended growth is used to inoculate 100 ml of Heart Infusion Broth containing 10% defibrinated rabbit blood, which is incubated as a stationary culture for approximately 18 hours at 37.degree. C..+-.2.degree. C. The 100 ml of liquified culture (working seed) is checked for purity by microscopic examination of a Gram-stained smear and growth on Heart Infusion Blood Agar plates. The working seed may be stored at 2.degree.-8.degree. C. for up to 14 days or used immediately. Two-liter Erlenmeyer flasks or other suitable vessels, containing Pneumococcus Inoculum Medium (YUF), containing dextrose (25 gm/liter), are inoculated with working seed and incubated stationary for approximately 8-24 hours at 37.degree. C..+-.2.degree. C. The incubation period varies as specified depending on the type of Streptococcus pneumoniae being grown. The pH of the fermentation is adjusted to maintain a target pH range of 6.0 to 7.2 by the periodic addition of 12% sodium bicarbonate solution until an optical density of 1.5 to 4.0 is reached. Optical density is monitored at 660 nanometers. A sample of the growth is examined microscopically and a serological agglutination reaction is performed to check purity. The growth from this stage is transferred into a seed fermenter containing 40 liters of Pneumococcus fermenter Medium composed of distilled water, a dry charge of the components for Pneumococcus seed medium (YUF), Yeast Extract Ultrafiltrate, UCON, and dextrose (approximately 25 gm/liter). The culture is incubated at 37.degree. C..+-.2.degree. C. with mild agitation for approximately 2-12 hours. The pH is controlled to 6.0 to 7.2 by the periodic addition of sodium hydroxide solution. A fermenter containing 525 liters of Pneumococcus fermenter Medium, composed of distilled water, a dry charge of the components for Pneumococcus Production Medium (YUF), Yeast Extract Ultrafiltrate, UCON and dextrose (approximately 25 gm/liter), is inoculated with approximately 50 liters of one 2-12 hour seed culture. The culture is incubated at 37.degree. C..+-.2.degree. C. with mild agitation for 6-30 hours depending on which type is being grown. The pH is controlled at 6.0 to 7.2 by periodic additions of sodium hydroxide solution. The fermentation is followed by optical density determination, and the fermentation is terminated when the dextrose is completely utilized as indicated by no further changes in pH.

Detailed Description Text (57):
S. pneumoniae 6B-OMPC Conjugate, Pn6B-Ps-OMPC:

Detailed Description Text (138):
Each Chinchilla was injected subcutaneously or intramuscularly with 0, 0.25, 1.0, or 4.0 .mu.g of Pn6B-Ps-OMPC adsorbed to A1(OH).sub.3. The Chinchillas were bled at 0,

2,4,6, and 8 weeks. The animals were challenged with Streptococcus pneumoniae 6B eight weeks after injection and monitored every 1-3 days by otoscopy and tympanometry. Middle ear effusions were aspirated for culture and the animals were sacrificed two weeks post challenge. The sacrificed animals were analyzed for middle ear histopathology. There was 60% mortality in animals receiving no conjugate while even the lowest dose resulted in 0% mortality. There was no protection against purulent otitis media in animals that did not receive conjugate while those receiving conjugate were protected at levels between 60 and 100% across all dosage ranges.

Detailed Description Text (196):
Pn-Ps-MIEP conjugates are capable of generating an immune response in mice consisting of IgG anti-Pn-Ps antibody and a memory response. This is in contrast to the Pn-Ps-CRM and Pn-Ps-DT which do not elict measurable anti-Pn-Ps antibody. Thus, MIEP functions as an immunologic carrier protein for Pn-Ps and is capable of engendering an anti-Pn-Ps antibody response when covalently conjugated to the Pn-Ps antigen. Purified MIEP is therefore an effective immunologic carrier protein replacing the heterogeneous OMPC in construction of bacterial polysaccharide conjugate vaccines.

Detailed Description Text (211):
S. Pneumoniae 18C-OMPC Conjugate, Pn18C-Ps-OMPC:

Detailed Description Text (213):
B. Preparation of S. Pneumoniae type 18C polysaccharide tetrabutylammonium form [Pn18C(Bu.sub.4 N.sup.+)]: A 60 mL column of Dowex 50.times.2 (Bu.sub.4 N.sup.+) was washed with 250 mL of H.sub.2 O. Pn18C-polysaccharide (m.w. reduced(650 mg) was covered with 65 mL of H.sub.2 O and stirred for 1 hr at which time all seemed to be in solution. This solution was applied to the column and allowed to percolate through by gravity (for 2 hr then under vacuum for 1 hr). The column was washed with 150 mL of H.sub.2 O and the combined effluents were lyophilized affording 655 mg of the 18C (Bu.sub.4 N.sup.+) salt. Twenty five mg was removed for nmr analysis and retained material.

Detailed Description Text (234):
S. Pneumoniae Type 4-OMPC Conjugate, Pn4-Ps-OMPC:

Detailed Description Text (236):
B. Preparation of S. Pneumoniae type 4 polysaccharide tetrabutylammonium form [Pn4 (Bu.sub.4 N.sup.+)]: A 65 mL column of Dowex 50.times.2 (Bu.sub.4 N.sup.+) was washed with 520 mL of H.sub.2 O. Pn 4-polysaccharide (m.w. reduced (400 mg) was covered with 35 mL of H.sub.2 O and stirred for 20 min at which time all seemed to be in solution

(stirring was continued overnight). This solution was applied to the column and allowed to percolate through by gravity and the column was washed with 150 mL of H.sub.2 O and the combined effluents were lyophilized.affording 504 mg of the Pn 4 (Bu.sub.4 N.sup.+) salt.

Detailed Description Text (289):
The results show that the retension of the side groups in the sized PnPs were approximately 90% for Pn9V-Ps and 80% for Pn4 and 18C. The retention of O-acetate in Pn1SC-Ps-OMPC conjugate aqueous bulk was found to be approximately 50%. The theoretical values for Pn18C-Ps and Pn4 are 1 mole of acetate or pyruvate per mole of Ps repeating unit and for Pn9V the ratio is 2:1. [Jansson, P-E., Lindberg, B., and Lindquist, U. `Structural studies of the capsular polysaccharides from Streptococcus pneumoniae Type 4.` Carbohyd. Res., 95:73-80, (1981). Lugrowski, C. and Jennings, H. J. `Structural determination of the capsular polysaccharide of Streptococcus pneumoniae Type 18C.` Carbohyd. Res. 131:119-129, (1984). Perry, M. B., Daoust, V., and Carlos, D. J. `The specific capsular polysaccharide of Streptococcus pneumoniae Type 9V.` Can. J. Biochem. 59:524-533, (1981)]. The lower retention of O-acetate found in the Pn1SC-Ps-OMPC conjugate is expected due to the susceptibility of O-acetyl groups hydrolysis to alkaline conditions at low temperatures.

Other Reference Publication (9):
H. Snippe et al., Infec. and Immunity, 42; No. 2, pp. 842-844 (1983). "Preparation of a Semisynthetic Vaccine to Streptococcus pneumoniae, Type 3".

CLAIMS:

1. A conjugate comprising an immunogenic protein selected from OMPC and MIEP covalently linked to a polysaccharide derived from one or more subtypes of Streptococcus pneumoniae, said polysaccharide having, on average, between 60 and 1200 repeating units per molecule and a polydispersity between 1.0 and 1.4, wherein said polysaccharide has a molecular weight between, on average, 1.times.10.sup.5 and 1.times.10.sup.6, and a level of contamination by pneumococcal group-specific C-polysaccharide below 3.0% of the type-specific polysaccharide.

4. The conjugate of claim 3 wherein said polysaccharide is derived from any of the subtypes of Streptococcus pneumoniae selected from: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F.

5. The conjugate of claim 4 wherein said polysaccharide is derived from between one and all of the capsular polysaccharides from Streptococcus pneumoniae subtype: 4, 6B, 9V,

14, 18C, 19F, or 23F.

6. The conjugate of claim 5 wherein said polysaccharide has a size polydispersity between 1.0 and 1.4, more than 60 repeating units per molecule, a C-polysaccharide contamination level as compared with type specific polysaccharide of less than 3%, and is derived from:

1) Streptococcus pneumonias 6B, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.60.+-.0.05;

c) a M.sub.W between about 3.times.10.sup.5 and 7.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 1000 repeating units per molecule on average;

2) Streptococcus pneumoniae 14, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 8.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.60.+-.0.05;

c) a M.sub.W between about 4.times.10.sup.5 and 1.times.10.sup.6 ; and

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 0.6 and 1.6; and

e) less than about 1200 repeating units per molecule on average;

3) Streptococcus pneumoniae 19F, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 1000 monomer repeating units per molecule, on average;

4) Streptococcus pneumonias 23F, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.54.+-.0.05;

c) a M.sub.W between about 4.times.10.sup.5 and 8.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.5 and 3.0; and

e) less than about 1000 monomer repeating units per molecule, on average;

5) Streptococcus pneumoniae 4, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 4.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 5.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 3.0; and

e) less than about 600 monomer repeating units per molecule, on average;

6) Streptococcus pneumonias 9V, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 3.times.10.sup.5 and 7.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 800 monomer repeating units per molecule, on average; or

7) Streptococcus pneumonias 18C, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.5 and 3.0. and

e) less than about 700 monomer repeating units per molecule, on average; or a mixture of any of these polysaccharides.

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Search notes

L2: Entry 425 of 425 File: USPT Jun 16, 1987

US-PAT-NO: 4673574
DOCUMENT-IDENTIFIER: US 4673574 A

TITLE: Immunogenic conjugates

DATE-ISSUED: June 16, 1987

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Anderson; Porter W.	Rochester	NY	14620	

US-CL-CURRENT: 424/194.1; 424/197.11, 424/236.1, 424/237.1, 424/238.1, 424/239.1, 424/240.1, 424/241.1, 424/244.1, 424/256.1, 424/831, 424/832, 530/350

CLAIMS:

I claim:

1. immunogenic conjugate comprising the reductve amination product of an immunogenic capsular polymer fragment having a chain length of from about 10 to about 30 monomeric units and a reducing end, which fragment is derived from the capsular polymer of a Streptococcus pneumoniae or Haemophilus influenzae bacterium, and a bacterial toxin or toxoid.

2. The immunogenic conjugate of claim 1, wherein the capsular polymer is immunogenic in mature humans and less immunogenic in infant humans.

3. The immunogenic conjugate of claim 1, wherein the reductive amination is performed in the persence of cyanoborohydride anions.

4. The immunogenic conjugate of claim 1, wherein the toxin or toxoid is diphtheria toxin or toxoid.

5. The immunogenic conjugate of claim 4, wherein the toxoid is CRM.sub.197.

6. The immunogenic conjugate of claim 1, wherein the toxin or toxoid is tetanus toxin or toxoid.

7. The immunogenic conjugate of claim 1, wherein the toxin or toxoid is a pseudomonas toxin or toxoid.

8. The immunogenic conjugate of claim 1, wherein the toxin or toxiod is a

staphylococcus toxin or toxoid.

9. The immunogenic conjugate of claim 1, wherein the toxin or toxoid is a streptococcus toxin or toxoid.

10. The immunogenic conjugate of claim 1, wherein the toxin or toxoid is pertussis toxin or toxoid.

11. The immunogenic conjugate of claim 1, wherein the toxin or toxoid is Escherichia coli toxin or toxoid.

12. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Haemophilus influenzae type b.

13. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 3.

14. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 6.

15. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 12.

16. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 14.

17. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 19.

18. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 23.

19. The immunogenic conjugate of claim 1, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 51.

20. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Haemophilis influenzae type b.

21. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 6.

22. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 14.

23. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 19.

24. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 23.

25. The immunogenic conjugate of claim 1, wherein the fragment is derived from the capsular polymer by oxidative cleavage.

26. The immunogenic conjugate of claim 1, wherein the fragment is derived from the capsular polymer by periodate.

27. The immunogenic conjugate of claim 1, wherein the fragment is derived from the capsular polymer by hydrolysis of a glycosidic linkage.

28. The immunogenic conjugate of claim 27, wherein the hydrolysis is accomplished enzymatically.

29. The immunogenic conjugate of claim 27, wherein the hydrolysis is accomplished chemically.

30. The immunogenic conjugate of claim 27, wherein the hydrolysis is accomplished by acid.

31. The immunogenic conjugate of claim 12, wherein the fragment elutes on a column of Bio-Gel P-10 at a Ve/Vo ratio of .ltoreq.1.08.

32. The immunogenic conjugate of claim 12, wherein the fragment elutes on a column of Bio-Gel P-10 at a Ve/Vo ratio of 1.09-1.38.

33. The immunogenic conjugate of claim 12, wherein the fragment elutes on a column of Bio-Gel P-10 at a Ve/Vo ratio of 1.39-1.99.

34. An immunogenic conjugate comprising a formalin treated reductive amination product of an immunogenic capsular polymer fragment having a chain length of from about 10 to about 30 monomeric units and a reducing end, which fragment is derived from the capsular polymer of a Streptococcus pneumoniae or Haemophilus influenzae bacterium, and a bacterial toxin or toxoid.

35. The immunogenic conjugate of claim 34, wherein the bacterial toxoid is diptheria toxoid.

36. The immunogenic conjugate of claim 35, wherein the Toxoid is CRM.sub.197.

37. The immunogenic conjugate of claim 34, wherein the bacterial toxin or toxoid is tetanus toxin or toxoid.

38. An immunogenic conjugate of (1) a PRP polysaccharide fragment having reducing terminal groups derived from the capsular polysaccharide of Haemophilus influenzae type b by selective acidic hydrolysis of a portion of the ribosyl ribitol linkages therein and (2) the diphtheria toxin protein CRM.sub.197.

39. The conjugate of claim 38 prepared by the reductive amination of the PRP fragment and protein.

40. The conjugate of claim 38 prepared by reductive amination in the presence of cyanoborohydride anions.

41. The conjugate of claim 38 wherein said PRP fragment elutes from a column of Bio-Gel P-10 at a Ve/Vo ratio of .ltoreq.1.08.

42. The conjugate of claim 38 wherein said PRP fragment elutes from a column of Bio-Gel P-10 at a Ve/Vo ratio of 1.09-1.38.

43. The conjugate of claim 38 wherein said PRP fragment elutes from a column of Bio-Gel P-10 at a Ve/Vo ratio of 1.39-1.99.

44. The conjugate of claim 38 wherein said PRP fragment elutes from a column of Bio-Gel P-10 at a Ve/Vo ratio of 2.0-2.4.

45. A vaccine that elicits effective levels of anti-capsular polymer antibodies in humans, comprising: the immunogenic conjugate of claim 1.

46. A method for actively immunizing humans against a bacterial pathogen having a capsular polymer, comprising: administering an effective amount of the vaccine of claim 45.

47. A vaccine that elicits effective levels of anti-PRP antibody formations in young warm-blooded mammals comprising an immunogenic amount of the conjugate of claim 41 and a pharmaceutically acceptable carrier.

48. A vaccine that elicits effective levels anti-PRP antibody formations in young warm-blooded mammals comprising an immunogenic amount of the conjugate of claim 42 and a pharmaceutically acceptable carrier.

49. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Streptoccoccus pneumoniae serotype 3.

50. The immunogenic conjugate of claim 5, wherein the bacterial pathogen is Streptococcus pneumoniae serotype 51.

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L2: Entry 397 of 425 File: USPT Apr 22, 1997

US-PAT-NO: 5623057
DOCUMENT-IDENTIFIER: US 5623057 A

TITLE: Pneumococcal polysaccharide conjugate vaccine

DATE-ISSUED: April 22, 1997

INVENTOR-INFORMATION:

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Burke; Pamela D.	Lansdale	PA		

US-CL-CURRENT: 530/404; 424/193.1, 424/194.1, 424/197.11, 424/234.1, 424/237.1, 424/241.1, 424/244.1, 424/256.1, 424/260.1, 530/403, 530/405, 530/406, 530/408, 530/409

CLAIMS:

what is claimed is:

1. A conjugate comprising an immunogenic protein selected from OMPC and MIEP covalently linked to a polysaccharide derived from one or more subtypes of Streptococcus pneumoniae, said polysaccharide having, on average, between 60 and 1200 repeating units per molecule and a polydispersity between 1.0 and 1.4, wherein said polysaccharide has a molecular weight between, on average, 1.times.10.sup.5 and 1.times.10.sup.6, and a level of contamination by pneumococcal group-specific C-polysaccharide below 3.0% of the type-specific polysaccharide.

2. The conjugate of claim 1 wherein said polysaccharide has an antigenicity index between 0.4 and 1.1.

3. The conjugate of claim 2 wherein said polysaccharide has an intrinsic viscosity between 0.6 and 3.0 dL/g and an antigenicity index of between 0.7 and 1.1.

4. The conjugate of claim 3 wherein said polysaccharide is derived from any of the subtypes of Streptococcus pneumoniae selected from: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F.

5. The conjugate of claim 4 wherein said polysaccharide is derived from between one and all of the capsular polysaccharides from Streptococcus pneumoniae subtype: 4, 6B, 9V, 14, 18C, 19F, or 23F.

6. The conjugate of claim 5 wherein said polysaccharide has a size polydispersity between 1.0 and 1.4, more than 60 repeating units per molecule, a C-polysaccharide contamination level as compared with type specific polysaccharide of less than 3%, and is derived from:

1) Streptococcus pneumonias 6B, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.60.+-.0.05;

c) a M.sub.W between about 3.times.10.sup.5 and 7.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 1000 repeating units per molecule on average;

2) Streptococcus pneumoniae 14, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 8.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.60.+-.0.05;

c) a M.sub.W between about 4.times.10.sup.5 and 1.times.10.sup.6 ; and

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 0.6 and 1.6; and

e) less than about 1200 repeating units per molecule on average;

3) Streptococcus pneumoniae 19F, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 1000 monomer repeating units per molecule, on average;

4) Streptococcus pneumonias 23F, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.54.+-.0.05;

c) a M.sub.W between about 4.times.10.sup.5 and 8.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.5 and 3.0; and

e) less than about 1000 monomer repeating units per molecule, on average;

5) Streptococcus pneumoniae 4, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 4.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 5.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 3.0; and

e) less than about 600 monomer repeating units per molecule, on average;

6) Streptococcus pneumonias 9V, said polysaccharide having:

a) a M.sub.N between about 3.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 3.times.10.sup.5 and 7.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.0 and 2.0; and

e) less than about 800 monomer repeating units per molecule, on average; or

7) Streptococcus pneumonias 18C, said polysaccharide having:

a) a M.sub.N between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

b) a K.sub.d (peak) of about 0.65.+-.0.05;

c) a M.sub.W between about 2.times.10.sup.5 and 6.times.10.sup.5 ;

d) an intrinsic viscosity in 0.1M sodium phosphate, pH 7.2, between 1.5 and 3.0. and

e) less than about 700 monomer repeating units per molecule, on average; or a mixture of any of these polysaccharides.

7. The covalent conjugate of claim 6 wherein the OMPC or MIEP and the Pn-Ps are linked through a spacer as shown by the formula: ##STR28## for linkagages through the polysaccharide hydroxyls, or ##STR29## in the case of polysaccharides bearing unblocked carboxyllic acid groups, wherein PRO represents OMPC or MIEP, and Pn-Ps represents a penumococcal polysaccharide.

8. The conjugate of claim 7 wherein the conjugate has a Pn-Ps:OMPC, or Pn-Ps:MIEP mass ratio between about 0.05 and 0.5, and upon hydrolysis and amino acid analysis yields a SCMHC/Lys ratio between 0.01 and 0.15.

9. A pneumococcal polysaccharide-immunogenic protein conjugate produced by the process of:

(a) Culturing a pneumococcus and isolating crude pneumococcal polysaccharide or solubilizing pneumococcal polysaccharide powder;

(b) Purifying and partially-hydrolyzing the polysaccharide of step (a) to an endpoint predetermined to generate a polysaccharide amenable to conjugation having no more than a 30% reduction of the polysaccharide's type-specific antigenicity as compared with the crude polysaccharide of step (a); and

(c) Conjugating the product of step (b) with OMPC or MIEP; wherein the pneumococcus cultured in step (a) is selected from one or more of the subtypes: 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, and further, wherein the Pn-Ps retains its antigenic integrity as measured by Ouchterlony double immunodiffusion or rate nephelometry assay using an anti-Pn-Ps type-specific antibody, said Pn-Ps prior to conjugation being physically sheared in a Gaulin press at a pressure between about 2000 and 15000 PSI or hydrolyzed by heating at 100.degree. C. for 24 hours or by sonicating, to a viscosity for a 1 mg/ml solution in 0.9M sodium chloride or K.sub.d (peak) endpoint as follows for each listed Pn-Ps subtype:

Pn-Ps Subtype	Target Endpoint Viscosity (centistokes)	Target Endpoint K.sub.d (peak)
Pn4-Ps	1.5-1.00	0.65 .+-. 0.05
Pn6B-Ps	1.3-1.00	0.60 .+-. 0.05
Pn6B-Ps	1.3-1.00	0.60 .+-. 0.05
Pn9V-Ps	1.3-1.00	0.65 .+-. 0.05
Pn14-Ps	1.1-0.95	0.60 .+-. 0.05
Pn18C-Ps	1.5-1.00	0.65 .+-. 0.05
Pn19F-Ps	1.3-1.00	0.65 .+-. 0.05
Pn23F-Ps	1.5-1.00	0.54 .+-. 0.05;

optionally followed by chromatographic or alcohol fractionation to select material having a polydispersity below 1.4.

10. A process for making a Pn-Ps-PRO conjugate which comprises:

a) Isolating crude pneumococcal polysaccharide, Pn-Ps;

b) i-Optionally purifying the crude Pn-Ps by ion exchange adsorption of impurities; ii-Partially-hydrolyzing or mechanically-shearing the crude Pn-Ps;

Fractionating the partially-hydrolyzed Pn-Ps according to size and purity;

d) Derivatizing the fractionated Pn-Ps, derived from one or more pneumococcal subtypes according to steps (a)-(c), to display pendant nucleophilic or electrophilic moieties;

e) Isolating Neisseria meningitidis b OMPC, or MIEP;

f) Functionalizing the OMPC or MIEP to exhibit reactive electrophilic or nucleophilic moieties;

g) Conjugating the polysaccharide of step (d) with the protein of step (f);

h) Capping the conjugate to remove residual functional groups; and

i) Isolating the conjugate product, wherein steps (b) and (c) further comprise:

(b) 1-Optionally, adsorbing onto Whatman DE52 anionic impurities at a solution pH of about 5; 2-Partially hydrolyzing the Pn-Ps in solution to an endpoint viscosity predetermined to diminish the Pn-Ps binding to anti-pneumococcal type specific antibody by no more than 30% as compared with crude Pn-Ps by:

1. heating at 50.degree. to 150.degree. C. for between 1 to 48 hours; or

2. sonicating for periods of 5 seconds to 5 minutes, depending on the power setting of the sonication probe, followed by periods of cooling and additional sonication; or

3. shearing in a Gaulin press at pressures between about 2000 and 15000 PSI; and

c) Fractionating the partially-hydrolyzed Pn-Ps according to size and purity wherein step (c) comprises:

Fractionating the hydrolyzed Pn-Ps and selecting a fraction having a molecular weight in the range between 1.times.10.sup.5 and 1.times.10.sup.6 by:

i-differential alcohol solubility using isopropanol at concentrations predetermined to precipitate the desired Pn-Ps size range, or

ii-fractionation on a size-exclusion liquid chromatography column capable of including and fractionating polysaccharides in the size range between 5.times.10.sup.4 and 1.times.10.sup.6 wherein the

endpoint for hydrolysis or shear is determined by viscometry of a 1 mg/ml solution in 0.1M sodium phosphate, pH 7.2, or chromatography for each of the listed polysaccharides according to the end-point for that subtype Pn-Ps:

Pn-Ps Subtype	Target Endpoint Viscosity (centistokes)	Target Endpoint K.sub.d (peak)
Pn4-Ps	1.5-1.00	0.65 .+-. 0.05
Pn6B-Ps	1.3-1.00	0.60 .+-. 0.05
Pn9V-Ps	1.3-1.00	0.65 .+-. 0.05
Pn14-Ps	1.1-0.95	0.60 .+-. 0.05
Pn18C-Ps	1.5-1.00	0.65 .+-. 0.05
Pn19F-Ps	1.3-1.00	0.65 .+-. 0.05
Pn23F-Ps	1.5-1.00	0.54 .+-. 0.05.

L2: Entry 394 of 425 File: USPT Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679352 A
TITLE: Synthetic Haemophilus influenzae conjugate vaccine

Brief Summary Text (4):
Haemophilus influenzae type b (Hib) is a major cause of bacterial meningitis in children under five years of age (refs. 1, 2). The literature references are identified at the end of this disclosure). The bacterium is protected from phagocytosis by a polysaccharide capsule that is a repeating polymer of polyribosyl ribitol phosphate (PRP). Antibodies induced against the capsular polysaccharide of the organism are protective (ref. 3). Effective conjugate vaccines in which PRP is linked to different carrier proteins such as diphtheria toxoid (PRP-D), tetanus toxoid (PRP-T), CRM 197 (HbOC) and the outer membrane protein of Neisseria meningitidis have been developed (refs. 4, 5). However, these conjugate vaccines do not protect against other invasive encapsulated H. influenzae type a and c strains and, more importantly, against non-encapsulated non-typeable H. influenzae strains that are one of the common causes of otitis media for which there is no vaccine. Therefore, the inclusion of selected non-encapsulated H. influenzae immunogens in current Hib vaccines is necessary to develop a universal Hi vaccine.

Brief Summary Text (17):
A yet another aspect of the present invention is directed towards the provision of a new generation of polyvalent vaccines comprising immunogenic synthetic PRP-peptide conjugates, and Hi antigens combined with other vaccines, such as DTP-polio, Neisseria meningitidis serotype A, B, C, abd W, and S. pneumoniae serotype 6B, 14, 19F and 23F.

Detailed Description Text (39):
In preferred embodiments of the present invention, the glycoconjugate technology can be generally utilized to prepare conjugate vaccines against pathogenic encapsulated bacteria. Thus, the glycoconjugate technology of the present inventions may be applied to vaccinations to confer protection against infection with any bacteria expressing potential protective polysaccharidic antigens, including Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli, Neisseria meningitidis, Salmonella typhi, Streptococcus mutans, Cryptococcus neoformans, Klebsiella, Staphylococcus aureus and Pseudomonas

aerogenosa.

L4: Entry 208 of 215 File: USPT Oct 15, 1996

US-PAT-NO: 5565204
DOCUMENT-IDENTIFIER: US 5565204 A

TITLE: Pneumococcal polysaccharide-recombinant pneumolysin conjugate vaccines for immunization against pneumococcal infections .

DATE-ISSUED: October 15, 1996

INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/244.1; 424/184.1, 424/193.1, 424/197.11, 536/105, 536/126

CLAIMS:

What is claimed is:

1. An immunogenic polysaccharide-protein conjugate obtained by reductive amination comprising (a) an oxidized polysaccharide derived from the capsular polysaccharide of Streptococcal pneumoniae (S. pneumoniae), and (b) the pneumolysin protein of S. pneumoniae which is expressed recombinantly, where said pneumolysin is not toxoided or is not produced by site-specific mutagenesis prior to conjugation with said oxidized polysaccharide.

2. The conjugate of claim 1, wherein the capsular polysaccharide of S. pneumoniae is derived from Type 14 or Type 18C.

3. The conjugate of claim 2, wherein the capsular polysaccharide of S. pneumoniae is derived from Type 18C.

4. The conjugate of claim 1, wherein the pneumolysin which is expressed recombinantly is expressed in E. coli.

5. The conjugate of claim 4, wherein the pneumolysin which is expressed recombinantly is expressed in the E. coli strain designated SCS1.

6. The conjugate of claim 5, wherein the pneumolysin which is expressed recombinantly is expressed in the E. coli strain designated SCS1, which harbors a plasmid selected from the group consisting of the plasmid designated pGEX-PL 18C (ATCC 69654) and the plasmid designated pGEX-PL 18C/20 (ATCC 69655).

7. The conjugate of claim 6, wherein the pneumolysin which is expressed recombinantly is expressed in the E. coli strain designated SCS1, which harbors a plasmid designated pGEX-PL 18C (ATCC 69654).

8. The conjugate of claim 6, wherein the pneumolysin which is expressed recombinantly is expressed in the E. coli strain designated SCS1, which harbors a plasmid designated pGEX-PL 18C/20 (ATCC 69655).

9. The conjugate of claim 1, wherein the recombinantly-expressed pneumolysin is first linked to a spacer prior to conjugation with the oxidized polysaccharide derived from the capsular polysaccharide of S. pneumoniae.

10. The conjugate of claim 9, wherein the spacer is selected from the group consisting of adipic acid dihydrazide (ADH) and 6-aminocaproic acid.

11. The conjugate of claim 10, wherein the spacer is ADH.

12. A vaccine comprising an immunogenic polysaccharide-protein conjugate obtained by reductive amination comprising (a) an oxidized polysaccharide derived from the capsular polysaccharide of S. pneumoniae, and (b) the pneumolysin protein of S. pneumonias which is expressed recombinantly, where said pneumolysin is not toxoided or is not produced by site-specific mutagenesis prior to conjugation with said oxidized polysaccharide.

13. The vaccine of claim 12 which further comprises one or more of an immunologically acceptable diluent, carrier or adjuvant.

14. The vaccine of claim 12 which comprises a mixture of at least two immunogenic conjugates with oxidized polysaccharides derived from capsular polysaccharides of different types of S. pneumoniae.

15. A method of eliciting an antibody response to the capsular polysaccharide of S. pneumoniae in warm-blooded animals, which comprises adminstering to said animals an immunogenic amount of the vaccine of claim 14.

16. A method of immunizing against S. pneumonias-caused disease in warm-blooded animals, which comprises administering to said animals the vaccine of claim 14 in an immunogenic amount by intramuscular, intraperitoneal or subcutaneous injection.

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L2: Entry 373 of 425 File: USPT Feb 2, 1999

US-PAT-NO: 5866132
DOCUMENT-IDENTIFIER: US 5866132 A

TITLE: Immunogenic oligosaccharide compositions

DATE-ISSUED: February 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Malcolm; Andrew J.	Edmonton			CA

US-CL-CURRENT: 424/193.1; 424/234.1, 424/243.1, 530/395, 530/403, 530/405

CLAIMS:

What is claimed is:

1. A composition comprising a conjugate, wherein each conjugate consists essentially of:

(a) at least one oligosaccharide hapten which retains at least one immunogenic epitope wherein each said oligosaccharide hapten has a multiple of repeat subunits; and

(b) a carrier which elicits a thymus dependent immune response in a subject, wherein said hapten is covalently coupled directly to said carrier and wherein said hapten-carrier conjugate is protectively immunogenic.

2. The composition of claim 1 wherein said hapten is an oligosaccharide of a bacterial or viral polysaccharide.

3. The composition of claim 1 wherein the presence of said immunogenic epitope is determined using inhibition ELISA.

4. The composition of claim 2 wherein said oligosaccharide is produced by acid hydrolysis of said polysaccharide.

5. The composition of claim 1 wherein said protective immunogenicity is determined by isotype ELISA.

6. The composition of claim 1 wherein said protective immunogenicity is determined by bactericidal or opsonization assay.

7. The composition of claim 2 wherein said polysaccharide is selected from the

group consisting of capsular polysaccharides of S. pneumococcus serotypes 1, 2, 3, 4, 5, 6B, 7, 7F, 8, 9N, 9V, 10A, 11A, 12, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22, 23F and 33F.

8. The composition of claim 1 which comprises two or more haptens.

9. The composition of claim 1 which does not induce carrier suppression.

10. The composition of claim 1 which does not induce antigenic competition.

11. The composition of claim 1 further comprising an adjuvant.

12. A composition comprising:

(a) a conjugate which comprises a size-separated oligosaccharide of S. pneumoniae serotype 8 which retains an immunogenic epitope which oligosaccharide is directly coupled to a protein carrier which elicits a thymus dependent immune response in a subject; and

(b) a suitable pharmaceutical excipient, wherein said conjugate provides an immunoprotective effect.

13. The composition of claim 12 which does not induce carrier suppression.

14. The composition of claim 12 which does not induce antigenic competition .

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